PYCR: Human Pyrroline-5-Carboxylate Reductase

PDB:2IZZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC001504
Entry Clone Source:MGC
SGC Clone Accession:
Tag:
Host:BL-21(DE3)R3 phage resistant

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*sMSVGFIGAGQLAFALAKGFTAAGVLAAHKIMASSPDMDLATVSALRKMGVKLTPHNKETVQHSD
VLFLAVKPHIIPFILDEIGADIEDRHIVVSCAAGVTISSIEKKLSAFRPAPRVIRCMTNTPVVVREGATVYATGTHAQVEDGRLMEQ
LLSSVGFCTEVEEDLIDAVTGLSGSGPAYAFTALDALADGGVKMGLPRRLAVRLGAQALLGAAKMLLHSEQHPGQLKDNVSSPGGAT
IHALHVLESGGFRSLLINAVEASCIRTRELQSMADQEQVSPAAIKKTILDKVKLDSPAG TAL

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Medium: TB + 50 µg/ml Kanamycin

10ml of overnight culture was added into 1L TB with 50mg/ml of Kanamycin (total 6L). The cells were cultured at 37°C until the OD reached 0.5 and then the temperature decreased to 18°C. IPTG was added to 0.5mM final concentration and the culture kept at 18°C overnight. The cells were collected by centrifugation and frozen at -80°C.

Purification

Procedure
Column 1 : Ni-Sepharose 6 Fast Flow
Buffers: Binding buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole.
Wash Buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 40 mM imidazole.
Elution Buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole.
Procedure: The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20 ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 8 ml of elution buffer.

Column 2 : Superdex 200 Hiload 16 60
Buffers : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP
Procedure: The eluted protein from the Ni-affinity column was loaded on the gel filtration column in GF buffer at 0.80 ml/min on an AKTA Purifier system. Eluted proteins were collected in 2 ml fractions.

Protein concentration: 37.15 mg/ml

Extraction

Procedure
The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer and then centrifuged at 37000 x g. The supernatant was kept for further purification.
Concentration:
Ligand
MassSpec: The mass determined was 33941 Da (33999 expected).
Crystallization: Crystals were grown by vapour diffusion at 20°C in 150 nl sitting drops. NADH to a final concentration of 5 mM was added to the protein just prior to crystallisation. The drops were prepared by mixing 100nl of protein solution and 50 nl of buffer consisting of 0.2 M Na2SO4, 0.1 M Bis-Tris pH 7.5, 20% PEG 3350 and 10% EtGly.Crystals were transferred to a cryo-protectant consisting of 20% EtGly, 80 % well solution before flash-cooling in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 1.95 Å; X-ray source: Synchrotron SLS -X10.
Data Processing: